

PATENT  
DOCKET NO.: 2026-4253US3

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant(s) : Cieplak, W.

Group Art Unit: 1814

Serial No. : 08/483,326

Examiner: Bugaisky, G.

Filed : June 7, 1995

For : PERTUSSIS TOXIN GENE: CLONING AND EXPRESSION

DECLARATION UNDER 37 C.F.R. §1.131

COMMISSIONER OF PATENTS AND TRADEMARKS  
Washington, D.C. 20231

Sir:

I, Witold Cieplak, Jr., am named as the inventor in the above indicated patent application, and I state as follows:

1. In a Declaration dated March 24, 1997, I stated that prior to July 1, 1988, the claimed invention was conceived and reduced to practice. In fact, the invention was conceived and reduced to practice even before September 1, 1987. The results of these first experiments showing the invention are described below.
2. The cloned gene and its expression product have the laboratory designation mutant 4-1. Mutant 4-1 possesses and exhibits the characteristics disclosed in Patent applications 07/311,612 and its continuation 07/542,149.
3. Exhibit pages 1-3 include laboratory notebook pages which demonstrate ADP-ribosyltransferase assays involving various pertussis toxin mutants, including a demonstration of substantially reduced enzyme activity associated with mutant 4-

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1. On the bottom of page 1, a brief outline of the ADP-ribosyltransferase assay is provided. The samples were incubated in the presence of the acceptor G protein transducin [*adenylate*- $^{32}\text{P}$ ]NAD $^{+}$  for 30 minutes at 37°C. The ADP-ribosyltransferase activity was measured as the extent of transfer of  $^{32}\text{P}$  from the radiolabeled NAD $^{+}$  to transducin. The amount of  $^{32}\text{P}$  incorporation into transducin was determined in two ways. First, the reaction samples were incubated with trichloroacetic acid (TCA) after the addition of bovine serum albumin to precipitate the proteins. The resultant TCA pellets were air dried after an ether wash and the amount of radioactivity in each pellet was determined by Cerenkov spectrometry to provide a quantitative estimate of ADP-ribosyltransferase activity. This assay revealed the lack of detectable transferase activity in the 4-1 mutant sample (labelled 4-1 on right side of table, labelled SAM #24 on left) compared to the other mutants and the positive control (labelled "PTX" on right side of table, labelled SAM #2 on left side). Second, the TCA precipitated proteins were solubilized in electrophoresis sample buffer and separated by sodium dodecylsulfate polyacrylamide gel electrophoresis. The gel was dried on filter paper and exposed to X-ray film. Page three is a copy of the resultant autoradiograph, showing that the reaction mixture containing the 4-1 mutant (fourth lane from the left) contained little or no detectable radiolabelled transducin (as evidenced by the lack of a band corresponding to 39 kDa) when compared to reaction mixtures containing other mutants or 6A-4, a wild type version of the S1 subunit. This assay confirmed the results of the quantitative analysis described above and demonstrates that mutant 4-1 has substantially reduced ADP-ribosyltransferase activity when compared to either pertussis toxin or other mutants.

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4. Exhibit pages 4-5 show a stained protein gel and three Western blots which demonstrate the reactivity of mutant 4-1 with a monoclonal antibody called "SATO" (also known as 1B7). The protein gel (bottom half of page 4) shows the presence of protein in all of the samples, while the Western blots demonstrate the selective recognition of the antibodies used. The blots labelled "R $\alpha$ PTX" represent the protein samples seen in the protein gel, as reacted with a rabbit anti-pertussis antibody, called "R $\alpha$ PTX". This antibody was a polyclonal antibody which reacted with both PTX (control) and the 4-1 mutant (compare right-most lane and left-most lane). Similarly, the protein samples were reacted with the SATO monoclonal antibody, as seen in the blot labelled "SATO" on the top half of page 5. In these samples, the antibody reacted with both PTX (control) and the 4-1 mutant (compare right-most lane and left-most lane). These pages provided the first data demonstrating reactivity of the 4-1 mutant with a protective monoclonal antibody.

5. The actual dates on laboratory notebook pages described in section 2-4 above have been blocked out. I state that each laboratory notebook page in section 2-4 above was dated prior to September 1, 1987.

6. The work corresponding to section 2-4 above was carried out by me or a technician working under my direction in the United States.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the

DOCKET NO.: 2026-4253US3

United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Date: 4-21-99

By: 

Dr. Witold Cieplak, Jr.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 40399/177/NIHD

In re patent application of

Jerry M. Keith

Serial No. 07/542,149

Group Art Unit: 1814

Filed: June 22, 1990

Examiner: G. Bugaisky

For: PERTUSSIS TOXIN GENE:  
CLONING AND EXPRESSION

DECLARATION OF WITOLD CIEPLAK, JR.

The Honorable Commissioner of  
Patents and Trademarks  
Washington, D.C. 20231

Sir:

I, Witold Cieplak, Jr. hereby declare that:

(1) I have read the declaration of Dr. Jerry Keith attached hereto as Appendix 1.

(2) The copies of notebook pages attached to that declaration are copies of pages from my own notebook, as I was the one who carried out the work recorded on those pages.

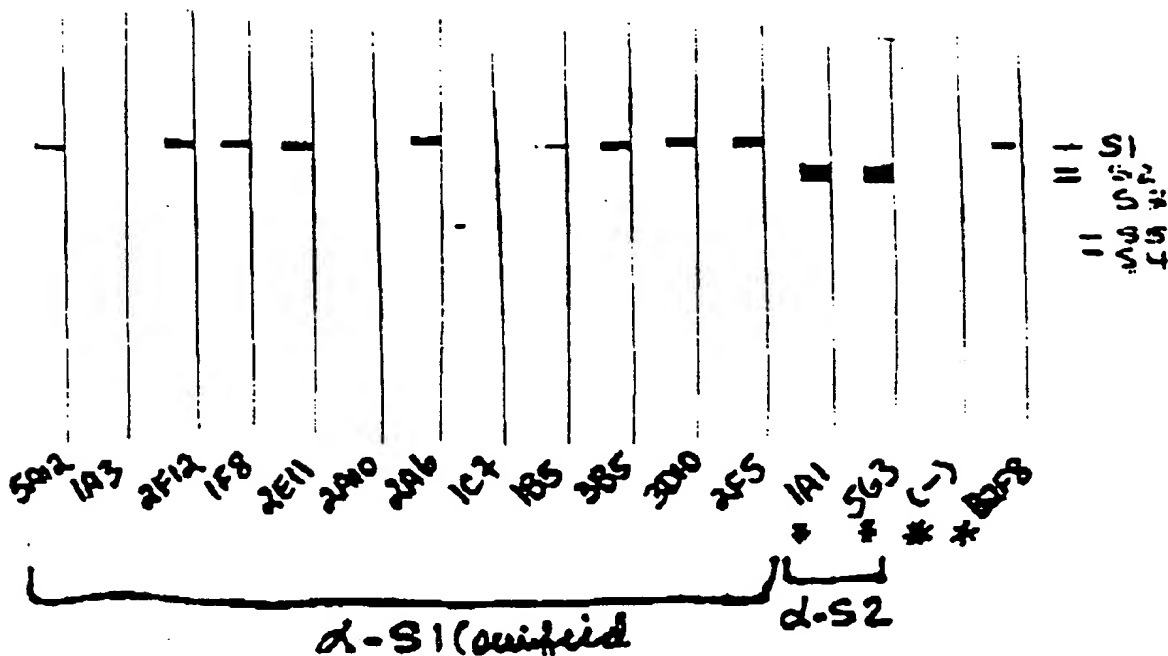
(3) I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

3/29/93  
Date

Witold Cieplak, Jr.  
Witold Cieplak, Jr.

EXHIBIT PAGE #2

PTX  
10ug



PTX 1A3 2F12 1F8 2E11 2A10 2A6 1C7 1B5 3B5 3D10 2F5 1A1 5G3 (-) B2F8

--✓-----=

Amgen mutant S1 protein  
monoclonal 1B7

12/17/91  
SENT BY:

18:18

301 402 0396

NIDR/LME

-17-91 : 2:32PM :

32-

301 402 0396: 5

EXHIBIT PAGE #3

Take samples (Average) - dilute 1:1 w/ water

Run ~ 25 ul for pattern (5 ul)  
~ 10 ul for water (2.5 ul total);

Dilution

(water)

1/2

- 1 Blank
- 2 10ul 300-Pat 2nd
- 3 PTx (5ul) - 5ul
- 4 6A
- 5 5-1
- 6 4-1
- 7 3-1 15ul
- 8 2-1 20ul
- 9 1-1
- 10 8-1
- 11 7-2
- 12 6-1
- 13 6A-2
- 14
- 15 0.2-1

Blank

Pat. Std (3ul)

PTx (2.5ul)

Same amount as 10ul

107

EXHIBIT PAGE #4

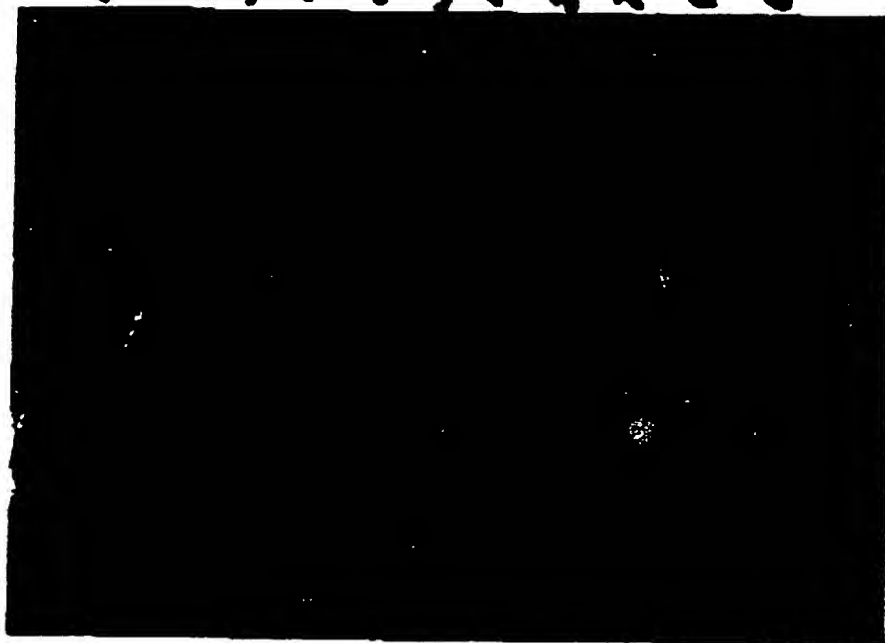
Protein Gel - Aragen nutrients in minimum bottles  
- 10 ml of 500mg/ml in 1x Lysosomal  
in 15mg ea (Sent by Akal)

Next time cut down on 1-1 abit for protein gel;

1-1 protein gel

PK (GND)

3-1 4-1 5-1 6-1 7-1 8-1 9-1 10-1 11-1 12-1





## EXHIBIT PAGE #5

also

For Western B(w/ new monoclonal) and Protein

DTX (1ug)	FOR 200ul of 1mg/ml in Laemmli			Need
	Sample	2X	1X	
pu C18	52.2	52.2	95.5	78.4 78.4 43
S13	<del>28.5</del>	<del>28.5</del>	<del>112</del>	- remove
Hsp8	33.7	33.7	132.5	too low
hsp 9	39.6	39.6	120.7	
Hsp 9	51.5	51.5	96.9	↓ actually
Ala 9	58.1	58.1	83.7	0.36
Det 9	56.6	56.6	26.8	amc
Hsp 8-9	48.0	48.0	103.8	
hsp 58	44.5	44.5	110.9	
Cel 41	33.1	33.1	133.7	
Sch 41	27.2	27.2	145.5	
Det 41	33.3	33.3	133.3	

Make 200ul samples of  $\text{pu}$  at 1mg/ml in Laemmli;

loaded (20ul = 20ug) into each lane of gel -

1 strip

1 blot

(Engel about pPTX13 in 1st moment)

removed: New S13 -	8umole	2X	1X
	28.43	78.43	43.1

Blotted 2 gels - pushed w/ 2F12 and 1B7

12-17-91 18:18  
SENT BY:

301 402 0398

NIDR/LME

77-17-91 : 2:37PM ;

32-

301 402 0396:# 8

EXHIBIT PAGE #6

137

2F12K

[REDACTED]



22/07/2022

フ

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力

# EXHIBIT PAGE # 8

324

3	369.70	577.10	21745.40	10.00	
4	442.30	577.10	22510.80	10.00	
5	450.30	5594.30	20702.80	10.00	
6	449.30	5592.00	24382.50	10.00	
7	130.70	1877.50	25768.10	10.00	2-1 ✓
8	163.50	2097.50	26200.70	10.00	
9	137.90	1585.50	5925.50	10.00	3-1 ✓
10	367.60	5671.50	8630.70	10.00	
11	396.20	5078.90	7625.40	10.00	
12	371.60	5389.20	20435.10	10.00	4-1 x
13	196.10	2095.20	24578.20	10.00	
14	287.20	4389.90	21945.50	10.00	
15	234.30	3437.70	12523.20	10.00	5-1
16	34.20	211.90	15388.10	10.00	
17	32.90	187.00	12737.90	10.00	
18	35.00	223.20	748.00	10.00	6-1 ✓
19	400.60	4613.40	754.60	10.00	
20	379.10	4982.40	762.40	10.00	
21	446.20	6774.50	27887.60	10.00	7-1 ✓
22	38.40	228.60	25621.90	10.00	
23	31.40	219.80	27576.10	10.00	
24	31.70	178.80	896.20	10.00	8-1 ✓
25	33.30	170.70	749.50	10.00	
26	32.00	178.20	649.30	10.00	
27	33.10	184.70	729.40	10.00	9-1 ✓
28	31.90	155.30	745.60	10.00	
29	39.00	268.20	797.40	10.00	
30	35.90	298.60	696.20	10.00	10-1 ✓
31	25.50	219.30	1092.50	10.00	
32	225.20	249.50	992.40	10.00	recout 792 1120A →
33	35.20	195.20	918.40	10.00	
34	37.70	257.30	809.90	10.00	
35			947.30	10.00	
36			348		
			963		

# EXHIBIT PAGE #9

(1) Construct (100mg) cpm $\pm$ SD *		upper limit
6A	25450 $\pm$ 950	26400
1-1	7393 $\pm$ 1367	8760
2-2	22319 $\pm$ 2096	24415
3-1	13549 $\pm$ 1596	<del>15145</del>
4-1	754 $\pm$ 7	761
5-1	26361 $\pm$ 1321	27682
6-1	764 $\pm$ 124	888
7-2	753 $\pm$ 30	783
8-1	926 $\pm$ 205	1131
first 20A	839 $\pm$ 68	907
FSI/1-4	952 $\pm$ 9	961

\* S.A. of 32P-NAO may have been a little on the low side.

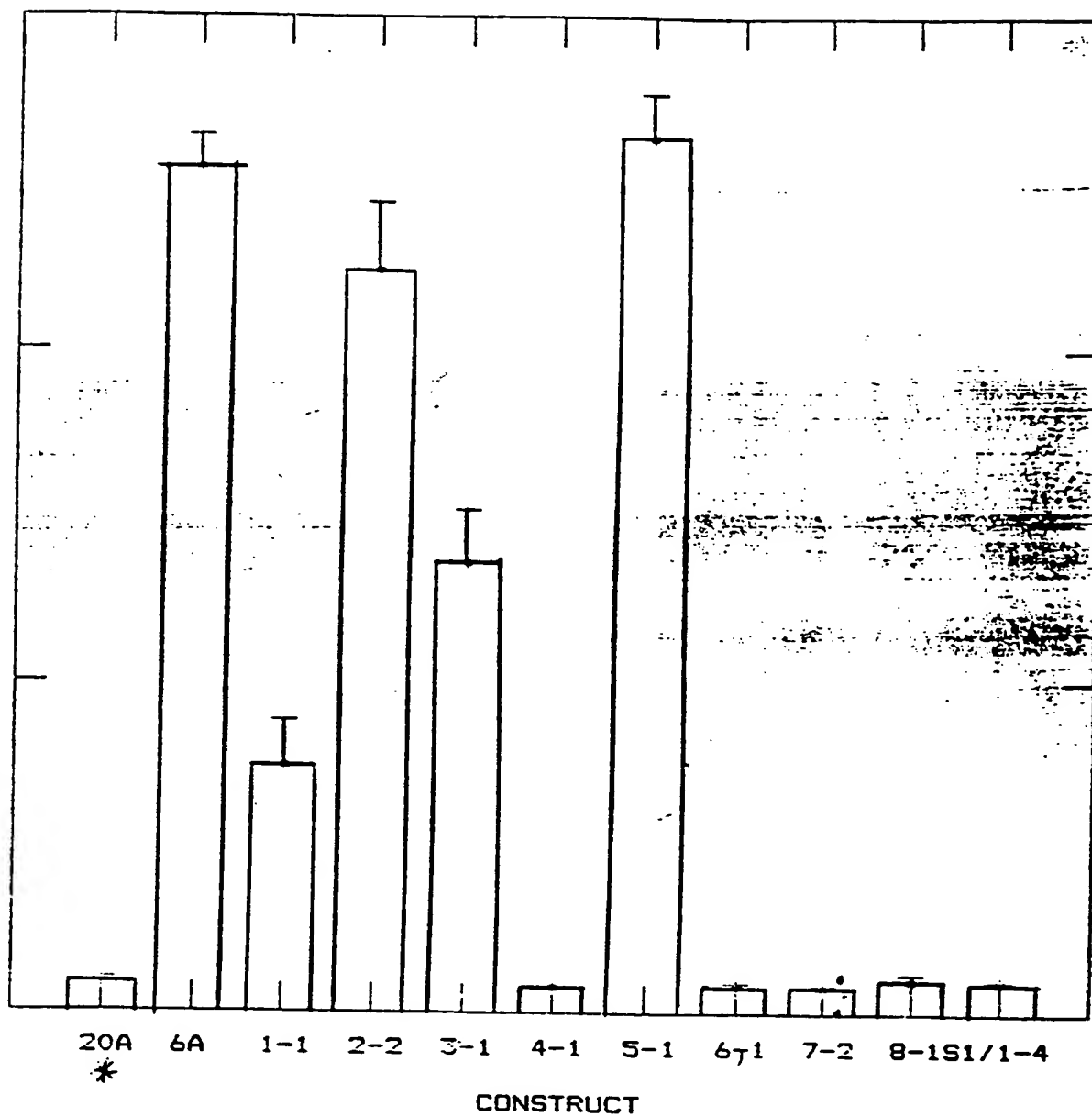
(1)

1/14

EXHIBIT PAGE #. 10

ADP-Ribosyltransferase Activity

TCA-PRECIPIITABLE CPM

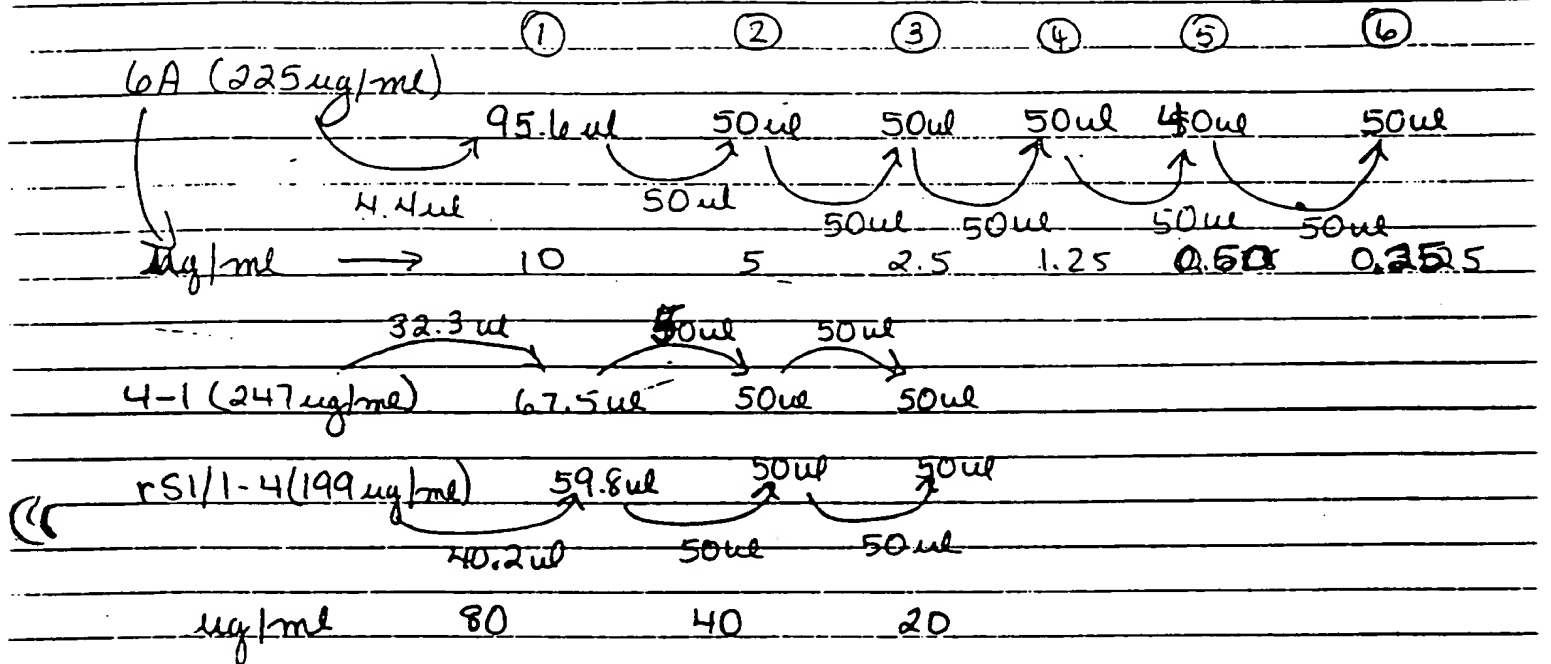


# EXHIBIT PAGE #11

ADP-ribosyl transferase activity: 20A, 6A, 4-1 and SI/1-4

1) All stocks frozen in 50 mM Tris HCl, pH 8.0 protein and densitometric scans already performed

2) Dilutions for assay: use Tris buffer:



3) Use 20 ul of each prep in 40 ul assay; assay 30' at 37°C w/ 4 ug/

U<sub>0</sub>

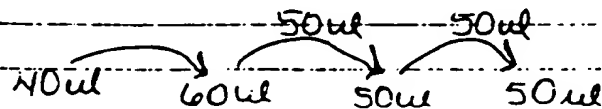
4) Final concentrations: (ug/ml)

6A 0.155, 0.3125, 0.625, 1.25, 2.5, 5.

4-1 } 10, 20, 40  
rSI/1-4 }

5) Reaction mixtures: 20 ul diluted  
10 ul 4X cocktail  
10 ul Translucin (400 ug/ml 50% glycerol)

N.B. 20A dilute



ENCLOSURE T PAGE # 12

DATE	DESCRIPTION	AMOUNT	BALANCE
1954	12/31	28.00	152.00
1955	1/1	101.00	152.00
1955	1/2	101.00	152.00
1955	1/3	101.00	152.00
1955	1/4	101.00	152.00
1955	1/5	101.00	152.00
1955	1/6	101.00	152.00
1955	1/7	101.00	152.00
1955	1/8	101.00	152.00
1955	1/9	101.00	152.00
1955	1/10	101.00	152.00
1955	1/11	101.00	152.00
1955	1/12	101.00	152.00
1955	1/13	101.00	152.00
1955	1/14	101.00	152.00
1955	1/15	101.00	152.00
1955	1/16	101.00	152.00
1955	1/17	101.00	152.00
1955	1/18	101.00	152.00
1955	1/19	101.00	152.00
1955	1/20	101.00	152.00
1955	1/21	101.00	152.00
1955	1/22	101.00	152.00
1955	1/23	101.00	152.00
1955	1/24	101.00	152.00
1955	1/25	101.00	152.00
1955	1/26	101.00	152.00
1955	1/27	101.00	152.00
1955	1/28	101.00	152.00
1955	1/29	101.00	152.00
1955	1/30	101.00	152.00
1955	1/31	101.00	152.00
1955	2/1	101.00	152.00
1955	2/2	101.00	152.00
1955	2/3	101.00	152.00
1955	2/4	101.00	152.00
1955	2/5	101.00	152.00
1955	2/6	101.00	152.00
1955	2/7	101.00	152.00
1955	2/8	101.00	152.00
1955	2/9	101.00	152.00
1955	2/10	101.00	152.00
1955	2/11	101.00	152.00
1955	2/12	101.00	152.00
1955	2/13	101.00	152.00
1955	2/14	101.00	152.00
1955	2/15	101.00	152.00
1955	2/16	101.00	152.00
1955	2/17	101.00	152.00
1955	2/18	101.00	152.00
1955	2/19	101.00	152.00
1955	2/20	101.00	152.00
1955	2/21	101.00	152.00
1955	2/22	101.00	152.00
1955	2/23	101.00	152.00
1955	2/24	101.00	152.00
1955	2/25	101.00	152.00
1955	2/26	101.00	152.00
1955	2/27	101.00	152.00
1955	2/28	101.00	152.00
1955	2/29	101.00	152.00
1955	2/30	101.00	152.00
1955	2/31	101.00	152.00
1955	3/1	101.00	152.00
1955	3/2	101.00	152.00
1955	3/3	101.00	152.00
1955	3/4	101.00	152.00
1955	3/5	101.00	152.00
1955	3/6	101.00	152.00
1955	3/7	101.00	152.00
1955	3/8	101.00	152.00
1955	3/9	101.00	152.00
1955	3/10	101.00	152.00
1955	3/11	101.00	152.00
1955	3/12	101.00	152.00
1955	3/13	101.00	152.00
1955	3/14	101.00	152.00
1955	3/15	101.00	152.00
1955	3/16	101.00	152.00
1955	3/17	101.00	152.

Blank (46)		
6A	200ng	45609
"	100ng	39883
"	50ng	37581
"	25ng	26686
"	10ng	13464
"	5ng	7588
20A	1.6 <del>ug</del>	39
"	0.8 <del>ug</del>	92.5
"	0.4 <del>ug</del>	102.5
$\left. \begin{array}{l} 39 \\ 92.5 \\ 102.5 \end{array} \right\} \bar{x} = 78$		
4-1	1.6 <del>ug</del>	149.5
"	0.8 <del>ug</del>	219
"	0.4 <del>ug</del>	62.5
<i>compared to buffer</i>		
SI/1-4	1.6 <del>ug</del>	238
"	0.8 <del>ug</del>	406.5
"	0.4 <del>ug</del>	312

4-1  $\frac{\% \text{ Control}}{\text{max}} = 0.01\%$   $\rightarrow$  5,000-fold decrease max.

5/1-4  $= 0.03\%$   $\rightarrow$  2,000-fold decrease.

1. 1. 1.

114



# EXHIBIT PAGE # 13

Sample	Final [ug/me]	$\bar{x}$ cpm	Net cpm
Buffer	<del>0.125</del> -	616	-
2A	0.125	8205	7589
"	0.250	14080	13464 *
"	0.625	27301	26685
"	1.25	38197	37581
"	2.5	42150	41534
"	5.0	46265	45649

20A	10.0 eqw	<del>708</del> 708	} x = <del>708</del> 78 cpm net
"	20.0 eq	708	
"	40 eqw	655	

				red factor
4-1	10	679	-39	> 5000
"	20	835	127	8481
"	40	765	110	> 10,000

SI/1-4	10	<del>928</del> 928	210	2564
"	20	<del>1023</del> 1023	315	3419
"	40	<del>1584</del> 1584	929	2318

these refer to processed protein content

EXHIBIT PAGE # 14

Series 4:22:47 PM

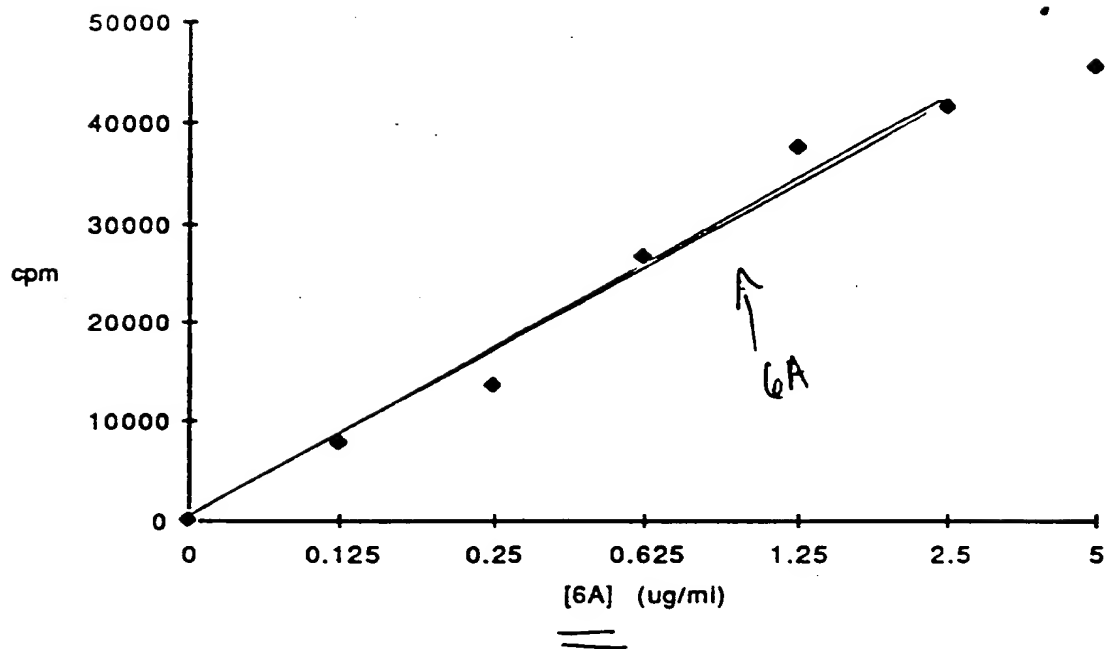
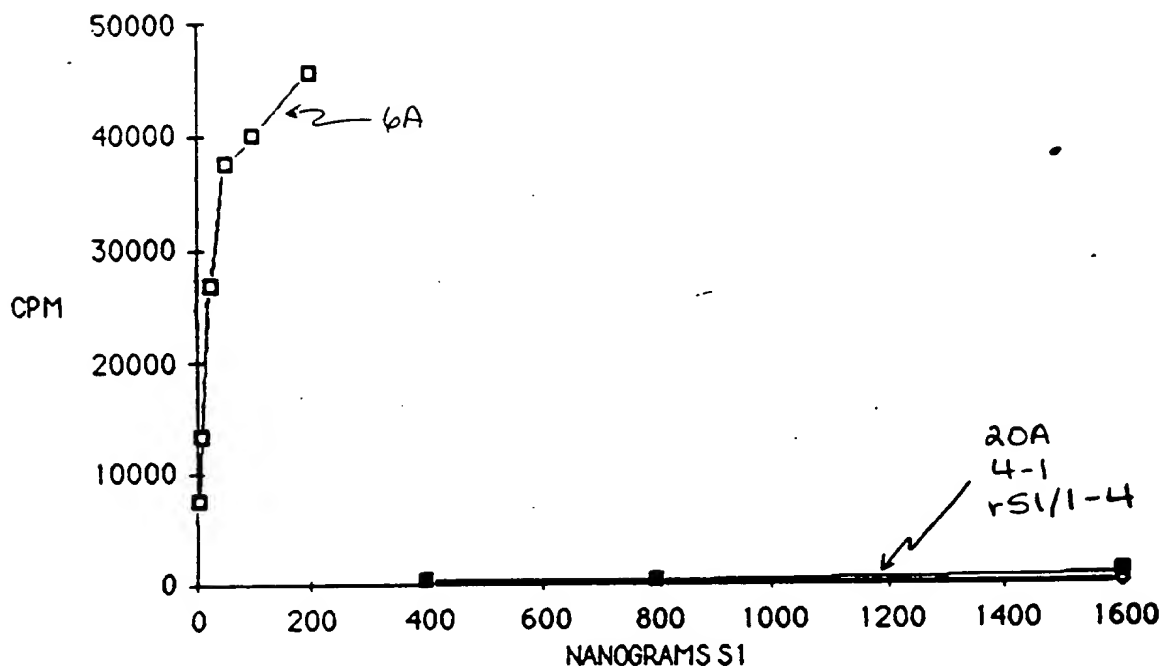


EXHIBIT PAGE #15



# VAD glycolipidase Assay

40 ug/ml in 100ul

6A	225	17.7	82.2
35A	69	57.9	42.1
39A	88	45.4	54.5
33B	113	35.3	64.7
2B	125	32.0	68.0
3B	157	25.4	74.6
1-1	75	53.3	46.6
2-2	75	↓	↓
3-1	75		
4-1	75		
5-1	75		
6-1	75		
7-2	75		
8-1	75		
20A	75	↓	↓

Assayed standard fraction in duplicate; 30°C for 4 hours

50 ug/ml = 1

$$\text{Cpm} \times 1.5 \times 1.5 \div 120 \div 76.9 \text{ sample}$$

EXHIBIT PAGE # 11

NAD glycohydrolase - used 20A glycol control  
all at 1 mg/assay

% control  $\pm$  S.D. (total)

6A	100
35A	$105.7 \pm 7.6$
39A	$35.3 \pm 2.3$
33B	$3.9 \pm 0.8$
2B	$1.6 \pm 1.5$
3B	$1.5 \pm 1.2$

1-1	$6.1 \pm 0.98$
2-2	$47.6 \pm 3.1$
3-1	$9.1 \pm 2.0$
4-1	$2.2 \pm 0.4$
5-1	$132.1 \pm 7.4$
6-1	$1.7 \pm 0.4$
7-2	$2.2 \pm 0.6$
8-1	$2.6 \pm 0.4$

New data

CL

EXHIBIT PAGE #18

1 ug/assay

NAD glycol hydrolase

20A (glycol)	805 $\pm$ 12	Net cpm	pmols rel/min/ug
6A	17,310 $\pm$ 701	16505	4.02
35A /	18,257 $\pm$ 1023	17452	4.25
39A /	6645 $\pm$ 304	5840	1.42
33B /	1452 $\pm$ 136	647	0.15
2B /	1072 $\pm$ 247	267	0.065
3B /	1062 $\pm$ 184	257	0.062
<del>20A (Trie)</del>	<del>1558 <math>\pm</math> 278</del>	-	-
1-1 /	1814 $\pm$ 156	1009	0.24
2-2 /	8670 $\pm$ 399	7865	1.9
3-1 /	2303 $\pm$ 329	1498	0.36
4-1 /	1175 $\pm$ 67	372	0.09
5-1 /	22,615 $\pm$ 796	21,810	5.3
6-1 /	1685 $\pm$ 70	280	0.068
7-2 /	1169 $\pm$ 102	364	0.088
8-1	1233 $\pm$ 59	428	0.10

[REDACTED]

TIME	TIME	TIME	TIME
5789.00	5577.00	5577.00	> 6A
5790.00	5578.00	5578.00	> 35A
5791.00	5579.00	5579.00	> 39A
5792.00	5580.00	5580.00	> 33B 1
5793.00	5581.00	5581.00	> 2B
5794.00	5582.00	5582.00	> 3B
5795.00	5583.00	5583.00	> -1-1
5796.00	5584.00	5584.00	> 2-2
5797.00	5585.00	5585.00	> 3-1
5798.00	5586.00	5586.00	> 4-1
5799.00	5587.00	5587.00	> 5-1
5800.00	5588.00	5588.00	> 6-1
5801.00	5589.00	5589.00	> 7-2
5802.00	5590.00	5590.00	> 8-1
5803.00	5591.00	5591.00	> 20A (Glycerol)
5804.00	5592.00	5592.00	
5805.00	5593.00	5593.00	
5806.00	5594.00	5594.00	
5807.00	5595.00	5595.00	
5808.00	5596.00	5596.00	
5809.00	5597.00	5597.00	
5810.00	5598.00	5598.00	
5811.00	5599.00	5599.00	
5812.00	5600.00	5600.00	
5813.00	5601.00	5601.00	
5814.00	5602.00	5602.00	
5815.00	5603.00	5603.00	
5816.00	5604.00	5604.00	
5817.00	5605.00	5605.00	
5818.00	5606.00	5606.00	
5819.00	5607.00	5607.00	
5820.00	5608.00	5608.00	
5821.00	5609.00	5609.00	
5822.00	5610.00	5610.00	
5823.00	5611.00	5611.00	
5824.00	5612.00	5612.00	
5825.00	5613.00	5613.00	
5826.00	5614.00	5614.00	
5827.00	5615.00	5615.00	
5828.00	5616.00	5616.00	
5829.00	5617.00	5617.00	
5830.00	5618.00	5618.00	
5831.00	5619.00	5619.00	
5832.00	5620.00	5620.00	
5833.00	5621.00	5621.00	
5834.00	5622.00	5622.00	
5835.00	5623.00	5623.00	
5836.00	5624.00	5624.00	
5837.00	5625.00	5625.00	
5838.00	5626.00	5626.00	
5839.00	5627.00	5627.00	
5840.00	5628.00	5628.00	
5841.00	5629.00	5629.00	
5842.00	5630.00	5630.00	
5843.00	5631.00	5631.00	
5844.00	5632.00	5632.00	
5845.00	5633.00	5633.00	
5846.00	5634.00	5634.00	
5847.00	5635.00	5635.00	
5848.00	5636.00	5636.00	
5849.00	5637.00	5637.00	
5850.00	5638.00	5638.00	
5851.00	5639.00	5639.00	
5852.00	5640.00	5640.00	
5853.00	5641.00	5641.00	
5854.00	5642.00	5642.00	
5855.00	5643.00	5643.00	
5856.00	5644.00	5644.00	
5857.00	5645.00	5645.00	
5858.00	5646.00	5646.00	
5859.00	5647.00	5647.00	
5860.00	5648.00	5648.00	
5861.00	5649.00	5649.00	
5862.00	5650.00	5650.00	
5863.00	5651.00	5651.00	
5864.00	5652.00	5652.00	
5865.00	5653.00	5653.00	
5866.			

Need ADP-ribosyltransferase

# EXHIBIT PAGE 3

NAD glycohydrolase Activity - Hanger mutants (New paper in Tris buffer)

2 hrs, 30°C, 30μM NAD

CPM (TOTAL)

Construct

0.25 μg

0.5 μg

1.0 μg

purchased SI from PTx

6,340 (1.54)

12,980.5 (3.16)

—

20A

F28

- 23 (0)

6A

1480.5 (0.36)

3,074 (0.75)

6446 (1.57)

1-1

73.5 (0.02)

254.5 (0.06)

486.5 (0.12)

2-2

562.5 (0.14)

1340 (0.33)

2734 (0.66)

3-1

125 (0.03)

419 (0.10)

882.5 (0.21)

4-1

31.5 (0.008)

-11 (0)(0)

34.5 (0.008)

5-1

1369 (0.33)

3011 (0.73)

6204 (1.51)

6-1

-5 0

-42 0

-30.5 0

7-2

-59 0

15 0

-58 0 ~~0.008~~

8-1

-5.5 0

-4 0

204 (0.05)

SI/1-4

-60 0

-99.5 0

-64 0

$$\text{cpm} \times \overset{\text{nmol}}{1.5} \times \overset{\text{ol}}{1.5} \times \overset{\text{bases}}{1.3} \div 100 \div \overset{\text{hrs}}{120} \div \mu\text{g}$$

$$= .0022437 \div \mu\text{g} = \text{units/min}$$



[REDACTED]

SRN	CPM1	CPM2	CPM3	TT4E
1	253.00	533.00	323.00	1.00
2	301.00	751.00	773.00	1.00
3	429.00	3445.00	2453.00	1.00
4	437.00	3554.00	3379.00	1.00
5	3456.00	13055.00	3062.00	1.00
6	3963.00	14300.00	14314.00	1.00
7	1750.00	5637.00	5347.00	1.00
8	3175.00	7437.00	7452.00	1.00
9	1777.00	7115.00	7122.00	1.00
10	1851.00	7170.00	7178.00	1.00
11	510.00	3808.00	3822.00	1.00
12	1553.00	3734.00	3745.00	1.00
13	956.00	2258.00	2256.00	1.00
14	379.00	2097.00	2104.00	1.00
15	432.00	1096.00	1104.00	1.00
16	523.00	1271.00	1286.00	1.00
17	435.00	978.00	991.00	1.00
18	3447.00	925.00	933.00	1.00
19	322.00	750.00	773.00	1.00
20	315.00	731.00	731.00	1.00
21	1299.00	3243.00	3250.00	1.00
22	446.00	3619.00	3630.00	1.00
23	802.00	1399.00	1399.00	1.00
24	513.00	2075.00	2082.00	1.00
25	113.00	1255.00	1272.00	1.00
26	243.00	1284.00	1272.00	1.00
27	111.00	1231.00	1245.00	1.00
28	111.00	1213.00	1235.00	1.00
29	133.00	1060.00	1075.00	1.00
30	111.00	1171.00	1181.00	1.00
31	111.00	1110.00	1141.00	1.00
32	111.00	815.00	830.00	1.00
33	111.00	731.00	730.00	1.00
34	111.00	732.00	730.00	1.00
35	111.00	730.00	730.00	1.00
36	111.00	730.00	730.00	1.00
37	111.00	730.00	730.00	1.00
38	111.00	730.00	730.00	1.00
39	111.00	730.00	730.00	1.00
40	111.00	730.00	730.00	1.00
41	111.00	730.00	730.00	1.00
42	111.00	730.00	730.00	1.00
43	111.00	730.00	730.00	1.00
44	111.00	730.00	730.00	1.00
45	111.00	730.00	730.00	1.00
46	111.00	730.00	730.00	1.00
47	111.00	730.00	730.00	1.00
48	111.00	730.00	730.00	1.00
49	111.00	730.00	730.00	1.00
50	111.00	730.00	730.00	1.00
51	111.00	730.00	730.00	1.00
52	111.00	730.00	730.00	1.00
53	111.00	730.00	730.00	1.00
54	111.00	730.00	730.00	1.00
55	111.00	730.00	730.00	1.00
56	111.00	730.00	730.00	1.00
57	111.00	730.00	730.00	1.00
58	111.00	730.00	730.00	1.00
59	111.00	730.00	730.00	1.00
60	111.00	730.00	730.00	1.00
61	111.00	730.00	730.00	1.00
62	111.00	730.00	730.00	1.00
63	111.00	730.00	730.00	1.00
64	111.00	730.00	730.00	1.00
65	111.00	730.00	730.00	1.00
66	111.00	730.00	730.00	1.00
67	111.00	730.00	730.00	1.00
68	111.00	730.00	730.00	1.00
69	111.00	730.00	730.00	1.00
70	111.00	730.00	730.00	1.00
71	111.00	730.00	730.00	1.00
72	111.00	730.00	730.00	1.00
73	111.00	730.00	730.00	1.00
74	111.00	730.00	7	

EXHIBIT F. 6E

NPS glycerophosphatase Activity Averages nutrients; <sup>duplicate</sup> ~~Experiment~~  
at 250, 500 and 1000 ng 21 June 30°C

pSI (2100 ug/ml)  
dilute to 40 ug/ml in 1:10 and 50 ul for (250) pt;  
① 15 ul + 135 ul 60 60  
(1000) (500) (250)

Nutrients: at 75 ug/ml

→ 9 → 100 100  
106.6 ul + 93.3 ml buff 100 100  
(1000) (500) (250)

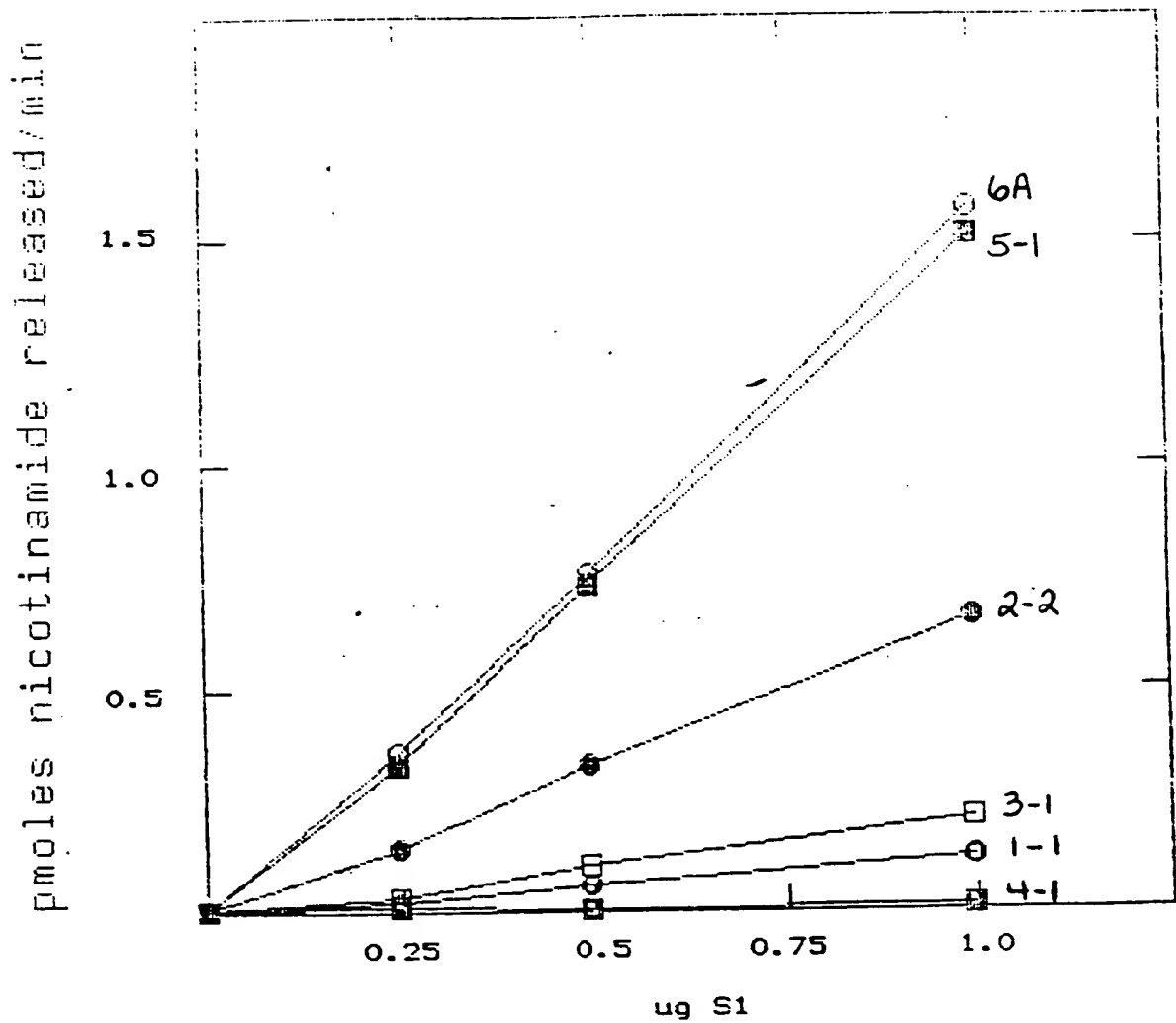


## EXHIBIT PAGE # 24

	0.25	0.5	1.0	% control
1eA	0.36	0.75	1.57	100
1-1	0.02	0.06	0.12	7.6
2-2	0.14	0.33	0.66	42.0
3-1	0.03	0.10	0.21	13.3
4-1	0.008	0.0	0.008	0.51
5-1	0.33	0.73	1.51	96.7
6-1		0	0	0
7-2		0	0.05	3.1
8-1		0	0	0

NAD Glycohydrolase Activity

EXHIBIT PAGE # 25



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket N . 40399/177/NIBD

In re patent application of

Jerry M. Keith

Serial No. 07/842,149

Group Art Unit: 1814

Filed: June 22, 1990

Examiner: G. Bugalsky

For: PERTUSSIS TOXIN GENE;  
CLONING AND EXPRESSION

DECLARATION OF WITOLD CISPLAK, JR.

The Honorable Commissioner of  
Patents and Trademarks  
Washington, D.C. 20231

Sir:

I, Witold Cisplak, Jr. hereby declare that:

1. I previously executed a declaration for this application. In my previous declaration, I stated that I carried out the experiments recorded on notebook pages attached to a declaration by Dr. Jerry Keith. A copy of that declaration by Dr. Keith was attached to my previous declaration as Appendix 1. With the exception of the notations on the top of each page regarding exhibit page numbers, the handwriting on all of those notebook pages is my handwriting.
2. At the time I performed these experiments, it was my practice to record my notes in a looseleaf notebook. Hence, there is no notebook cover bearing my name or table of contents page reflecting those experiments.
3. During the course of my research at Rocky Mountain Laboratories, NIAID (Hamilton, Montana), I conceived that a mutation at the arginine 9 position of the amino acid sequence of the S1 subunit of Bordetella pertussis toxin could yield a substantially detoxified mutant comprising an epitope that contributes to

immunoprotection against *Bordetella pertussis* toxicity. I subsequently discovered that such a mutation at the arginine 9 position in fact yielded a substantially detoxified mutant comprising an epitope that contributes to immunoprotection against *Bordetella pertussis* toxicity.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

12/11/93  
Date

Witold Cieplak, Jr.  
Witold Cieplak, Jr.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Attorney Docket No. 40399/177/NIHD

In re patent application of

Jerry M. Keith

Serial No. 07/542,149

Group Art Unit: 1814

Filed: June 22, 1990

Examiner: G. Bugaisky

For: PERTUSSIS TOXIN GENE:  
CLONING AND EXPRESSION

DECLARATION OF JERRY M. KEITH

The Honorable Commissioner of  
Patents and Trademarks  
Washington, D.C. 20231

Sir:

I, Jerry M. Keith, hereby declare that:

1. I have reviewed the Declaration of Dr. Cieplak attached hereto. I believe all statements in that declaration to be correct.

I further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

December 21, 1993  
Date

Jerry M. Keith  
Jerry M. Keith



Serial Number: 07/542,149

-2-

Art Unit: 1814

Claims 11, 13 and 15-16 are allowable. Prosecution is now closed.

The amendment to the specification is entered, as it is clear that an inadvertent error in sequencing of the deposited parental strain occurred. The amendment does not constitute new matter.

5 The change in inventorship is permissible. It does not appear necessary to revive parent application 07/311,612 in order to grant priority (MPEP § 201.3 re continuing applications). There is, however, now no continuity between this application and 06/843,727 (Patent No. 4,883,761).

10 All claims are allowable. However, due to a potential interference, *ex parte* prosecution is SUSPENDED FOR A PERIOD OF 3 MONTHS FROM THE DATE OF THIS LETTER.

Upon expiration of the period of suspension, applicant should make an inquiry as to the status of the application.

15 Any inquiry concerning this communication or earlier communications from the examiner should be directed to Gabriele E. Bugaisky, Ph.D. whose telephone number is (703) 308-4201.

20 Papers related to this application may be submitted to Group 180 by facsimile transmission. Papers should be faxed to Group 180 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM-1 Fax Center numbers are (703) 308-4227 and (703) 305-3014.

Any inquiry of a general nature or relating to the status of this application should be directed to the Group receptionist whose telephone number is (703) 308-0196.



ROBERT A. WAX  
SUPERVISORY PATENT EXAMINER  
GROUP 180

  
GEB

April 27, 1994

25

# Curie counts of TCA pellets.

PAGE

USER: 2 ID: SURVEY PRESET TIME: 1.00  
 SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR: N RS232 IN  
 H#: 0 AQ: N QCE: N YRCM: N  
 CHANNEL 1-LL: 0 UL: 400 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:  
 CHANNEL 2-LL: 0 UL: 670 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:  
 CHANNEL 3-LL: 0 UL: 1000 2SIGMA: 2.00 BKG SUB: 0.00 BKG 2SIG: 0.00 LSR:  
 DATA CALC: CTS, UNKNOWN REPLICATES: 1 NORM FACTOR: Q 1.00000  
 HALF-LIFE (DAYS): N

SAM	CTS1	CTS2	CTS3	TIME	
1	51.00	62.00	62.00	1.00	Buffer
2	3968.00	4089.00	4089.00	1.00	OTx (0.1ug)
3	315.00	347.00	347.00	1.00	S13 sup (8ug)
4	3560.00	3670.00	3670.00	1.00	S13 pellet (8ug)
5	421.00	494.00	494.00	1.00	S11 sup (8ug)
6	709.00	729.00	729.00	1.00	S11 pellet (8ug)
7	2857.00	2950.00	2950.00	1.00	6A-4#2
8	3071.00	3157.00	3157.00	1.00	6A-4#4
9	2354.00	2431.00	2431.00	1.00	6A-4#6
10	2271.00	2361.00	2361.00	1.00	6A-4#8
11	2126.00	2161.00	2161.00	1.00	Angen rS1 (0.8ug)
12	2982.00	3086.00	3086.00	1.00	6A-3
13	3195.00	3294.00	3294.00	1.00	35A
14	1253.00	1298.00	1298.00	1.00	39A
15	110.00	120.00	120.00	1.00	33B
16	76.00	89.00	89.00	1.00	2B
17	184.00	195.00	195.00	1.00	3B
18	87.00	97.00	97.00	1.00	14B
19	249.00	258.00	258.00	1.00	21B?
20	309.00	322.00	322.00	1.00	43B?
21	718.00	755.00	755.00	1.00	1-1
22	2087.00	2170.00	2170.00	1.00	2-2
23	781.00	818.00	818.00	1.00	3-1
24	73.00	85.00	85.00	1.00	4-1
25	3334.00	3458.00	3458.00	1.00	5-1
26	395.00	405.00	405.00	1.00	6-1?
27	407.00	418.00	418.00	1.00	6A-2?
28	372.00	377.00	377.00	1.00	7-2?
29	789.00	803.00	803.00	1.00	8-1?

Assay: 10ul 4X ADPR cocktail  
 20ul test sample (100ug/ml - 400ug/ml)  
 10ul Transducin (x0.7ug)  
 Stock

↓  
 0.5HR 37°C  
 ↓  
 Inc 20ug/ml BSA (next use OVA + 50ul in 10ul of 1-5 Bmg/ml)  
 ↓  
 50ul in 10ul 10% TCA  
 ↓  
 V.O.N. 40  
 ↓  
 Inc 50% TCA x2  
 1ml Ether  
 aspirate to dryness count

ADP-ribosyl transferase  
2nd gel -

Lane

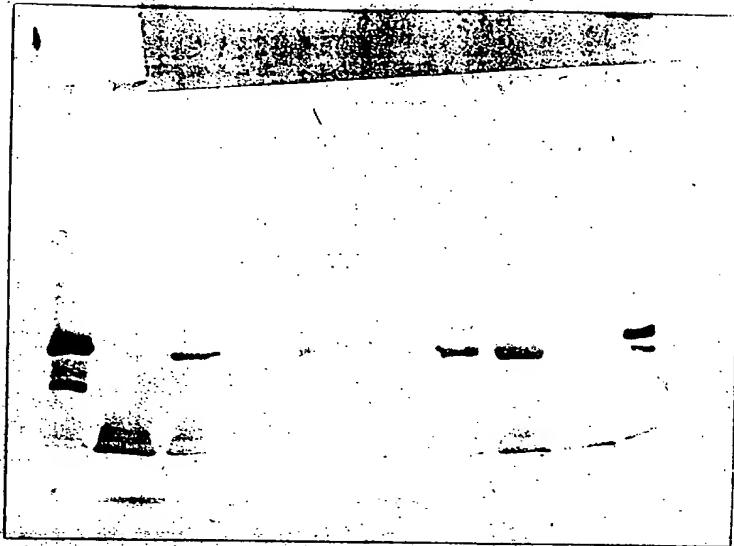
1	<del>Blank</del>
2	1 Bro-Rad
3	2 1-1
4	3 2-2
5	4 3-1
6	5 4-1
7	6 5-1
8	7 6-1
9	8 6A-2
10	9 7-2
11	10 8-1
12	11 6A-4#2
13	12 6A-4#4
14	13 6A-4#6
15	14 6A-4#8
15	

39K  
↓

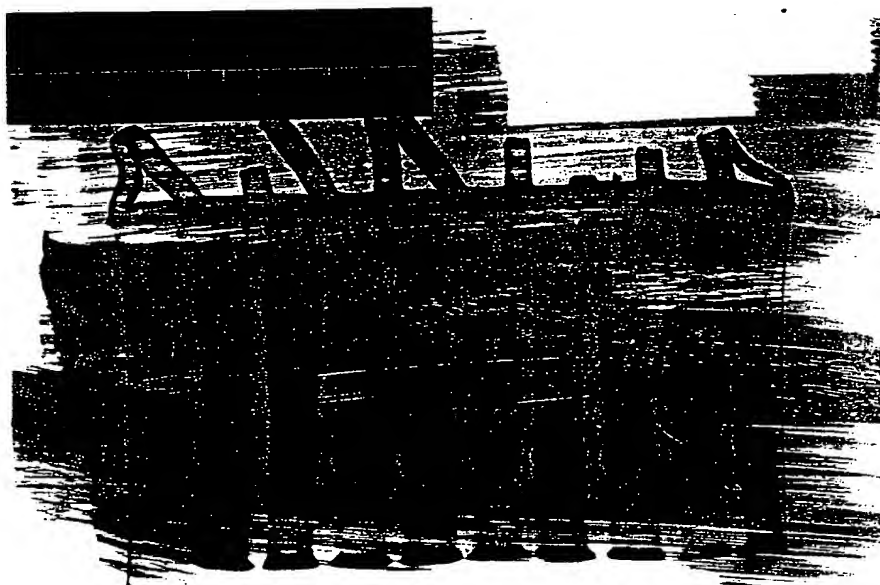
2-2 3-1 4-1 5-1 6-1 6A-2 7-2 8-1

6A-4

Ra ptx

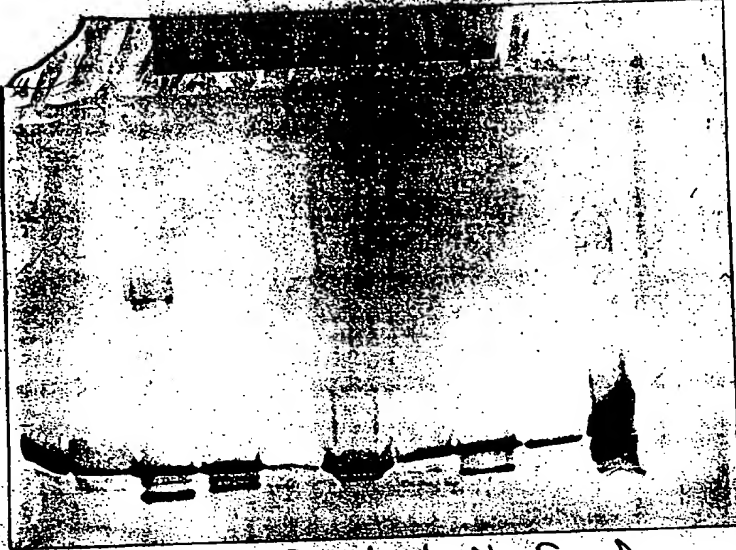


ptx  
8-1  
5-1  
1-1  
6-1  
3-1  
7-2  
2-2  
4-2  
4-1



4-1  
4-2  
7-2  
2-2  
3-1  
6-1  
1-1  
5-1  
8-1  
ptx

SATO



4-1  
6A-2  
2-2  
7-2  
3-1  
6-1  
1-1  
6-1  
8-1  
4-1

← this is the band

Raptor



4-1  
6A-2  
2-2  
7-2  
3-1  
6-1  
1-1  
6-1  
8-1  
4-1

TABLE 2

Complete Nucleotide Sequence of Pertussis Toxin Gene

**EcoRI**  
**GAATTC**GTGGCTGGCCCTGGTTCGCCGTATGGCCCCCAAGGGAACCGACCCCAAGATA  
 100  
 ATCGTCTGTCAACCGCCACATCAACGAGGCGCTGCAGTCCAAGGCGGTCTGCGAGGCC  
 TTTGCCGCCCAGGCGCCACGCCGGTCATCGCCACGCCGATCAGACCCGCGGCTTCATC  
 200  
 GCAGACGAGATCCAGCGCTGGGCGGGCGTCTGTGCGGAAAACCGGCGCCAAGCTGAAGTAG  
 300  
 CAGCGCAGCCCTCCAACCGGCCATCCCCGTCCGGCCGGCACCATCCCGCATACGTGTTGG  
 CAACCGCCAACCGGCATGCGTGCAGATTCTGCTACAAAACCTCGATTCTTCCGTACAT  
 400  
 CCGGCTACTGCAATCCAACACGGCATGAACGCTCCTTCGGCGCAAAGTCGCGCGATGGTA  
 CCGGTCACCGTCCGGACCGTCTGACCCCTGCCATGGTGTGATCCGTAATAAGGCAC  
 500  
**EAT**CAAAACGCAGAGGGGAAGACGGGATGCGTTGCACTCGGCAATTGCGCAAACCGCAA  
 ↑ (M R C T R A I R Q T A)  
 600  
 GAACAGGCTGGCTGACGTTGGCTGGCGATTCTTGGCGTACGGCGCCCGTGAATTCGCCCGG  
 R T G M L T W L A I L A V T A P V T S P  
 CATGGGCGGACGATCCTCCCGCCACCGTATACCGCTATGACTCCCGCCCGCGGAGGACG  
 A W A N D D P P A T V Y R Y D S R P P E D  
 700  
 TTTCCAGAACGGATTACCGCGTGGGGAACAACGACAATGTGCTCGACCATCTGACCG  
 V F Q N G F T A M G N H D H V L D H L T  
 GACGTTCTGCCAGGTCCGACAGCAACACGCGTTTCGTCTCCACCAGCAGCAGCCGGC  
 G R S C Q V G S S H S A F V S T S S S R  
 800  
 GCTATACCGAGGTCTATCTCGAATCGCATGCAGGAAGCGGTGAGGCGCAACCGCGCCG  
 R Y T E V Y L E H R H Q E A V E A E R A  
 900  
 GCAGGGGCACCGGCACTTCATCGGCTACATCTACGAAGTCCGCGCCGACAACAATTCT  
 G R G T G H F I G Y I Y E V R A D N H F  
 ACGGCGCGGCGCAGCTCGTACTTCGAATACGTCGACACTTATGGCGACAATGCCGGCCGT  
 Y G A A S S Y F E Y V D T Y G D N A G R  
 1000  
 TCCTCGCCCGCGCGCTGGCCACCTACCAGAGCGAATATCTGGCACACCGGCGCATTCGGC  
 I L A G A L A T Y Q S E Y L A H R R I P  
 CCGAAAACATCCGCGAGGTAAACCGGGTCTATCACAACGGCATCACCGGCGAGACCACGA  
 P E N I R R V T R V V H H G I T G E T T  
 1100  
 CCACGGAGTATTCCAACGCTCGGTACGTCAGCCAGCACTCTCGCGCCAATCCCAACCCCT  
 T T E Y S H A R Y V S Q Q T R A N P N P  
 1200  
 ACACATCGCGAAGGTCCGTAGCGTCGATCGTGGCACATTGGTGGCGATGGCGCCGGTGATAG  
 Y T S R R S V A S I V G T L V R H A P V I

TABLE 2  
Complete Nucleotide Sequence of Pertussis Toxin Gene

CGCCTTGCA<sup>1</sup>TGGCGCGGCA<sup>2</sup>GCGCGAAAGC<sup>3</sup>TCGAGGCCA<sup>4</sup>TGGCAGCCTG<sup>5</sup>GTCCGAACGC<sup>6</sup>  
S A C H A R Q A E S S E A M A A W S E R  
CGCGCGAGG<sup>7</sup>CGATGGTTCT<sup>8</sup>CGTGTACTAC<sup>9</sup>GAAAGCAT<sup>10</sup>CGCGTATT<sup>11</sup>CGTTCTAGACCTGG<sup>12</sup>  
A G E A H V L V Y Y E S I A Y S F U  
CCAGCCCCG<sup>13</sup>CCCAACTCCGGTAATTGAACAGCAT<sup>14</sup>GCGCGATCGACCGCAAGACGCTCTGC<sup>15</sup>  
M P I D R K T L C  
ATCTCCTGT<sup>16</sup>CCGTTCGCGTTGGCCCTC<sup>17</sup>CTCGGATCTC<sup>18</sup>ACGTGGCGCGGCGCCTCCACGC<sup>19</sup>  
H L L S V L P L A L L G S H V A R A S T  
CAGGCATCG<sup>20</sup>TATTCCGCCCGCAGGAACAGATTACCCAGCAT<sup>21</sup>GGCAGCCCCATGGACCGT<sup>22</sup>  
P G I V I P P Q E Q I T Q H G S P Y G R  
GCGCGAACAAGACCCCGTGC<sup>23</sup>CTGACCGTGC<sup>24</sup>CGGAATTGCGCGCGCAGCGGCGATCTGCAGG<sup>25</sup>  
C A N K T R A L T V A E L R G S G D L Q  
AGTACCTGC<sup>26</sup>GTATGTGACCGCGCGGTGGTCAATATTGCGCTCTACGATGGCACCTATC<sup>27</sup>  
E Y L R H V T R G W S I F A L Y D G T Y  
TCGGCGGCGAATATGGCGGCGT<sup>28</sup>GATCAAGGACGGAACACCCGGCGCGCGCATTCGACCTGA<sup>29</sup>  
L G G E Y G G V I K D G T P G G A F D L  
AAACGACGT<sup>30</sup>CTGCATCATGACCACGCGCAATACGGGTCAACCCGCAACCGATCACTACT<sup>31</sup>  
K T T F C I H T T R N T G Q P A T D H Y  
ACAGCAACG<sup>32</sup>TACCGCCACTCGCCTGCTCTCCAGCACCAACAGCAGGCTATGCGCGGTCT<sup>33</sup>  
Y S H V T A T R L L S S T N S R L C A V  
TCGTCAGAA<sup>34</sup>CGCGGCAACCGGTCATTGGCGCGCTGCACCA<sup>35</sup>CGCGTATGACGGCAAGTACT<sup>36</sup>  
F V R S G Q P V I G A C T S P Y D G K Y  
GGAGCATGT<sup>37</sup>ACAGCGCGCTGCGGAAATGCTTTACCTGATCTACGTGGCGCGCATCTCCG<sup>38</sup>  
W S H Y S R L R K H L Y L I Y V A G I S  
TACCGTCCAT<sup>39</sup>GTGAGCAAGGAAGAACAGTATTACGACTATGAGGACGCAACGTTCCGAG<sup>40</sup>  
V R V H V S K E E Q Y Y D Y E D A T F E  
CTTACGCCC<sup>41</sup>TACCGGCATCTCCATCTGCAATCCTGGATCATCCTTATGCTGAGACGCTT<sup>42</sup>  
T Y A L T G I S I C H P G S S L C U  
CCCCACTCGAACCACCGCCCGGGACAG<sup>43</sup>GGCGCGCGCGCGGTGCGCGCTGCGCGCCCT<sup>44</sup>  
M R A L  
GGCGTGGT<sup>45</sup>TGCTGGCATCCGCGCGGATGACGCATCTTTCCCCCGCCCTGGCGGACGTTCC<sup>46</sup>  
A W L L A S G A M T H L S P A L A D V P  
TTATGTGCT<sup>47</sup>GGTGAAGACCAATATGGTGGGTACCAAGCCTAGCCATGAAGCCGTATGAAGT<sup>48</sup>  
Y V L V K T N H V V T S V A H K P Y E V



TABLE 2

Complete Nucleotide Sequence of Pertussis Toxin Gene

CACCCCGACGCGCATGCTGGTCTGCGGCAICGCCGCCAACTGGGCGCCGCGGCCAGCAG  
I P T R M L V C G I A A K L G A A A S S  
2300  
CCCGGACGCGCACGTGCCGTTCTGCTTCGGCAAGGATCTCAAGCGTCCCGGCAGCAGTCC  
P D A H V P F C F G K D L K R P G S S P  
2400  
CATGGAAGTCATGTTGCGCGCCGTCTTCAIGCAACAACGGCCGCTGCGCATGTTTCTGGG  
M E V M L R A V F M Q Q R P L R M F L G  
TCCCAAGCAACTCACTTTTGAAGGCAAGCCCGCGCTCGAAGTATCCGGATGGTTCGAATG  
P K Q L T F E G K P A L E L I R M V E C  
CAGCGGCAAGCAGGATTGCCCTGAAGGCGAACCCTCATGATACCATCGCATCCATCCTG  
S G K Q D C P U FM H T I A S I L  
TTGTCCGTGCTCGGCATATACAGCCCGGCTGACGTGCGCGGCTTGCCGACCCATCTGTAC  
L S V L G I Y S P A D V \*A G L P T H L Y  
2600  
AAGAACTTCACTGTCCAGGAGCTGGCCTTGAACTGAAGGGCAAGAATCAGGAGTTCTGC  
K N F T V Q E L A L K L K G K N Q E F C  
2700  
CTGACCGCCCTTCATGTGCGGGCAGAAGCCTGGTCCGGGCGTGCTGTCCGACGCGGGACAC  
L T A F M S G R S L V R A C L S D A G H  
GAGCAGGACACGTGGTTCGACACCATGCTTGGCTTTGCCATATCCGCGTATGCGCTCAAG  
E H D T W F D T M L G F A I S A Y A L K  
2800  
AGCCGGATCGCGCTGACGGTGGAAGACTCGCCGTATCCGGGCACTCCCGGCGATCTGCTC  
S R I A L T V E D S P Y P G T P G D L L  
GAACTGCAGATCTGCCCGCTCAACGGATAITGCGAATGAACCCTTCCGGAGGTTTCGACG  
E L Q I C P L N G Y C E U  
2900  
TTTCCGCGCAATCCGCTTGAGACGATCTTCCGCCCTGGTTCATTCCGGGAACACCGCAA  
[53]→ 3000  
CATGCTGATCAACAACAAGAAGCTGCTTCATCACATTCTGCCATCCTGGTGCTCGCCCT  
FM L I N N K K L L H H I L P I L V L A L  
GCTGGGCATGCGCACGGCCCAGGCCGTTGCGCCAGGCATCGTCATCCCGCCGAAGGCACT  
L G M R T A Q A \*V A P G I V I P P K A L  
3100  
GTTACCCCAACAGGGCGGCGCCTATGGACGCTGCCCGAACGGAACCCGCGCCTTGACCGT  
F T Q Q G G A Y G R C P N G T R A L T V  
GGCCGAAGTGGCGGCAACGCCGAATTGCAGACGTATTTGCGCCAGATAACGCCCGGCTG  
A E L R G H A E L Q T Y L R Q I T P G W  
3200  
GTCCATATACGGTCTCTATGACGGTACGTACCTGGGCCAGGCGTACGGCGGCATCATCA  
S I Y G L Y D G T Y L G Q A Y G G I I K  
3300  
GGACGCGCCGCCAGGCGCGGGGTTCAITTTATCGCGAAACTTTCTGCATCAGGACCATATA  
D A P P G A G F I Y R E T F C I T T I Y

TABLE 2

Complete Nucleotide Sequence of Pertussis Toxin Gene

```

CAAGACCGGGCAACCGGCTGCGGATCACTACTACAGCAAGGTCACGGCCACGGCGCTGCT
K T G Q P A A D H Y Y S K V T A T R L L
                                     3400
CGCCAGCACCAACAGCAGGCTGTGCGCGGTATTCGTCAGGGACGGGCAATCGGTCATCGG
A S T N S R L C A V F V R D G Q S V I G
AGCCTGCGCCAGCCCGTATGAAGGCAGGTACAGAGACATGTACGACGCGCTGCGGCGCCT
A C A S P Y E G R Y R D M Y D A L R R L
                                     3500
GCTGTACATGATCTATATGTCCGGCCTTGCCGTACCGGTCCACGTCAGCAAGGAAGAGCA
L Y M I Y M S G L A V R V H V S K E E Q
                                     3600
GTATTACGACTACGAGGACGCCACATTCCAGACCTATGCCCTCACCGGCATTTCCTCTG
Y Y D Y E D A T F Q T Y A L T G I S L C
CAACCCGGCAGCGTCGATATGCTGAGCCGCGCGCTCGGATCTGTTCCCTGTCCATGTT
N P A A S I C U
                                     3700
TTCCTTGACGGATAACCGGAATGAATCCCTTGAAAGACTTGAGAGCATCGCTACCGCGCC
TGGCCTTCATGGCAGCCTGCACCCTGTTGTCCGCCACGCTGCCCGACCTCGCCCAGGCCG
                                     3800
GCGGCGGGCTGCAGCGCTGTCAACCACTTCATGGCGAGCATCGTGGTCTGTACTGCCCGGG
                                     3900
CGGTCAGTGGCCACGGTGACCATCGCCATAATCTGGGCGGGCTACAAGCTGCTGTTCCGG
CACGCCGATGTGCTGGACGTGGTGCGTGTGGTGCTGGCGGGAGCTGCTGATCGGCGCATC
                                     4000
GGCCGAAATCGCTCGTTATCTGCTGACCTGAATCCTGGACGTATCGAACATGCGTGATCC
GCTTTTCAAGGGCTGCACCCGGCGCGCGGATGCTGATGGCGTACCCGCCACGGCAGGCCG
                                     4100
TGTGCAGCCGGCACCATTCCCTGCTGGGCCATCTCGGTTACGATCCGCTTTCTGGCCTT
                                     4200
GTTTCCCGTGCGCATTGCTGGCGATCGGATCATGATCCGGCGGATGACCAGCAGTTCCG
Sau3A
CCTGATC

```

The deduced amino acid sequences of the individual subunits are shown in the single letter code below the nucleotide sequence. The proposed signal peptide cleavage sites are indicated by asterisks. The start of the protein coding region for each subunit is indicated by the box and arrow over the initiation codon. Putative ribosomal binding sites are underlined. The promoter-like sequence is shown in the -35 and -10 boxes. Proposed transcriptional start site is indicated by the arrow in the CAT box. Inverted repeats are indicated by the arrows in the flanking regions.

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